University of Manitoba

the Children's Hospital Research Institute of Manitoba
a division of the Children's Hospital Foundation of Manitoba

DREAM

Diabetes Research Envisioned and Accomplished in Manitoba

Presents

7th Annual Diabetes Research Symposium

Wed. November 7th – Thurs. November 8th, 2018

Wednesday November 7th, 2018

9:00am	**Poster Setup, self-registration** (Pick up name badge and program from registration table) *Joe Doupe Concourse – second floor Basic Medical Sciences Building (outside Theatre A)*		
9:45am – 10:00am	**Trainee Day Opening Remarks – DREAM Trainee Executive** ***Theatre A***		
10:00am – 11:30am	**Trainee Workshop:** *"Transferable skills: learn to talk about what you do instead of what you know"* *Beyond the Professorate -* **L. Maren Wood** ***Theatre A***		
11:30am – 1:00pm	**Poster Competition & Lunch** – Preliminary Round *(Winners to present in Guided Poster Session Thursday at 12:30pm)* *Joe Doupe Concourse (2nd floor Basic Medical Sciences Building)* **Note to Poster Presenters, please be at your posters at the following times:** 12:00pm – 12:30pm: Even poster numbers 12:30pm – 1:00pm: Odd poster numbers		
1:00pm – 2:00pm	**iCARE Study Showcase** – *open to all registered attendees* The iCARE (improving renal complications in adolescents with type 2 diabetes through research) study was designed to address the extremely high rates of early kidney damage in youth with type 2 diabetes. This study has recently expanded to include national cohorts, and the revised objectives are: 1) Characterize the primary bio-psycho-social (BPS) risk factors associated with prevalent and progressive albuminuria, 2) Determine individual, family and community level factors that influence biological and psychological risk factors and behaviours (adherence) that could be modified to protect against prevalent and progressive albuminuria, 3) Determine if systemic renal inflammation is the common pathway through which BPS risk factors lead to albuminuria in youth with type 2 diabetes. ***Theatre A***		
2:00pm – 3:00pm	**Trainee Workshop:** ***Theatre A*** *"Developing your academic CV"* **Dr. Sachin Katyal** *Assistant Professor, Department of Pharmacology and Therapeutics, University of Manitoba*	**2:00pm – 5:00pm**	**iCARE National Meeting** *(Closed Meeting for invited PIs and Trainees only)* ***Brodie 405***
3:00pm – 3:30pm	**Coffee Break**		
3:30pm – 4:30pm	**Trainee Workshop:** ***Theatre A*** *"Turning your CV into a resume for non-academic careers"* **Kate Yee** *Career Consultant, Career Services, University of Manitoba*		
4:30 – 5:00pm	**Trainee Workshop:** ***Theatre A*** *"Writing a strong cover letter"* **Dr. Afshin Raouf** *Associate Professor, Department of Immunology University of Manitoba*		
8:00pm	**Pub Night –** *off Campus* *8pm until late* ***Yellow Dog Tavern*** *– 386 Donald St.*		

Thursday November 8th, 2018

Pediatric Grand Rounds: *Chair: Dr. Brandy Wicklow*	***Theatre A***
8:00am – 9:00am	***5th Annual Dr. Heather Dean Lecture for Excellence in Pediatric Diabetes Research:*** **Dr. Louise Maple-Brown,** *Charles Darwin University, Australia* ***"A life-course approach to address the challenges of youth-onset type 2 diabetes in Australian Aboriginal communities"***
9:00am – 9:30am	Coffee, light breakfast, registration, poster set-up ***Joe Doupe Concourse (2nd floor BMSB)***
Morning Session: *Chairs: Drs. Elizabeth Sellers & Vern Dolinsky*	***Theatre C***
9:30am – 9:45am	**Opening Ceremony and Opening Prayer** ***Anishinaabe Elders (Pine Creek First Nation):*** **Barbara and Clarence Nepinak**
9:45am – 10:00am	**Welcome from DREAM Co-Directors and Dedication to Mary Jane Wood and Bertha Flett**
10:00am – 11:00am	**Dr. Jill Hamilton,** *Hospital for Sick Children, Toronto* ***"Sweet Babies: Outcomes of Gestational Diabetes Exposure In utero"***
11:00am – 12:00pm	**Dr. Tim Kieffer,** *University of British Columbia* ***" Cell-Based Insulin Replacement for Diabetes "***
12:00pm – 1:15pm	**Lunch & Guided Poster Session** - *Joe Doupe Concourse (2nd floor BMSB)* Guided Poster Session: The top 3 Posters from the Wednesday poster session will present a second time in this guided poster session **selected presenters will be notified by email immediately after the poster session on Wednesday* 12:30pm - 12:40pm *Speaker #1 TBD* 12:40pm - 12:50pm *Speaker #2 TBD* 12:50pm - 1:00pm *Speaker #3 TBD*
Afternoon Session: *Chairs: Drs. Jon McGavock & Christine Doucette*	***Theatre C***
1:15pm – 1:30pm	**Melissa Gabbs**, *University of Manitoba* ***High rates of ambulatory and nocturnal hypertension in youth with type 2 diabetes***
1:30pm – 1:45pm	**Stephanie Kereliuk**, *University of Manitoba* ***Exposure to gestational diabetes alters cardiac gene expression and metabolism in the rat offspring and induces cardiac dysfunction with age***
1:45pm – 2:45pm	**Dr. Elaine Urbina,** *Cincinnati Children's Hospital* ***"Vascular and Cardiac Target Organ Damage in Diabetes"***
2:45pm – 3:15pm	Coffee Break ***Joe Doupe Concourse (2nd floor BMSB)***
3:15pm – 4:15pm	**Dr. Joseph Bass,** *Northwestern University, Chicago (Eli Lilly-Sponsored Keynote)* ***"Circadian Integration of Glucose Homeostasis With Sleep/Wake State"***
4:15pm – 4:30pm	**Simone da Silva Rosa**, *University of Manitoba* ***Therapeutic targeting of skeletal muscle nix in early-onset insulin resistance***
4:30pm – 4:45pm	**Myriam Hoyeck**, *Carleton University* ***Effects of chronic low-dose TCDD exposure during pregnancy on offspring pancreas development and function; a potential risk factor for increased diabetes susceptibility***
7:00pm	**Banquet Dinner & Awards** *Rudy's Eat & Drink – 375 Graham Ave* **for registered attendees only*

WELCOME!

GREETINGS FROM THE CHRIM SCIENTIFIC DIRECTOR

Welcome to the 7th Annual DREAM Diabetes Research Symposium!

The DREAM Diabetes Symposium has quickly become a key event for researchers, medical professionals, trainees and others to learn the latest on diabetes research taking place here in Manitoba and around the world. Every year, the DREAM symposium continues to evolve and grow. In addition to presentations from world-renowned keynote speakers, the DREAM symposium offers a forum for our trainees to share their work with the wider Manitoban community. This year, the organizers received 18 abstracts for poster presentations, 4 of which were selected by the organizing committee to present their work to you today in a short talk. Additionally, the symposium will kick off with the 5th Annual *Heather Dean Lecture in Excellence in Diabetes* where we will hear a presentation by Dr. Louise Maple-Brown from Darwin University in Australia. Enjoy the day and knowledge sharing as we continue to conduct research that will improve the lives of young people living with Diabetes.

Terry Klassen, MD
CEO and Scientific Director
CHRIM

GREETINGS FROM THE DREAM CO-DIRECTORS

Welcome to the 7th Annual Diabetes Research Envisioned and Accomplished in Manitoba (DREAM) Symposium. This year we are pleased to coordinate our meeting with the CIHR-funded national iCARE Network Team, focused on reducing the burden of renal complications in youth living with type 2 diabetes. Members of the scientific team and patient council will be joining us for the 2-day symposium. We are pleased to once again host an outstanding group of international and national speakers that span the continuum of research from basic discoveries to clinical investigation and community participatory care/research. We are pleased to partner with the Department of Pediatrics on the 5th annual Dr. Heather Dean Lecture in Excellence in Diabetes Research. This year's speaker, Dr. Louise Maple-Brown will be visiting from Australia to present the exciting work being done to improve the lives of Aboriginal Youth living with and at risk for type 2 diabetes.

As we have done for the past few years the DREAM trainees have organized an exciting day of professional development and scholarly work to support trainees from across the province. Please take time Wednesday and Thursday November 7th and 8th to take time during the breaks to view the exciting work of our trainees and network with the 5 internationally renowned speakers. We hope that you will enjoy this symposium and that you will come away with a new appreciation of the forms of community- and patient-oriented research that are currently underway in the realm of diabetes in youth. We are deeply indebted to the hard work and dedication from Jana Slaght and Christine Doucette who, once again, put this wonderful symposium together!

Vern Dolinsky and Jon McGavock
Co-Directors, DREAM

DR. HEATHER DEAN LECTURE

IN EXCELLENCE IN DIABETES

The 6th Annual DREAM Symposium marks the 4th Annual Dr. Heather Dean Lecture in Excellence in Diabetes. This lecture was named in honour of one of the University of Manitoba's most recognized and trailblazing clinician scientists. Dr. Dean has been a pillar in several communities in our beloved province for nearly 40 years, including but not limited to the medical community, the pediatrics and child health community, the farming community, the sporting community and most famously, the knitting community. Dr. Dean has inspired countless trainees, patients, families and athletes during her tenure in the province. The DREAM team thought it was important to name the opening lecture for our symposium in Dr. Dean's name as without her dedicated commitment to diabetes in children and vision for team-based care, the DREAM team would not exist. The Annual Dr. Heather Dean Lecture in Excellence in Diabetes will symbolize the excellence in clinical care, research and interdisciplinary collaboration in the area of pediatric endocrinology that Dr. Dean has embodied and cultivated in the province of Manitoba. We hope that the lecture will also serve as an annual source of inspiration for young hearts and minds in the same way that Dr. Dean has inspired us over the past 30 years.

DR. LOUISE MAPLE-BROWN MBBS, FRACP, PhD.

"A life-course approach to address the challenges of youth-onset type 2 diabetes in Australian Aboriginal communities"

In the context of the escalating epidemic of chronic diseases among Indigenous Australians, it is vital that we reduce risk as early as possible in the life course of an individual. We have developed a partnership between researchers, health care providers and policy organisations in Australia's Northern Territory and North Queensland, to address the issues of intergenerational diabetes and diabetes in pregnancy in the high-risk population of these regions. We are working with health service providers to optimise antenatal care and to develop strategies to improve maternal health pre-conception and between pregnancies, particularly among young women with type 2 diabetes themselves. The PANDORA Study (Pregnancy and neonatal diabetes outcomes in remote Australia), a longitudinal birth cohort, sits within our Diabetes in Pregnancy Partnership, and involves over 1100 NT women and their babies.

BIOGRAPHY

Dr. Louise Maple-Brown is Head of Department of Endocrinology, Royal Darwin Hospital (Northern Territory, Australia) and an NHMRC Practitioner Fellow with Menzies School of Health Research. Louise leads a clinical research program within the Wellbeing and Preventable Chronic Diseases division of Menzies, with a focus on diabetes and related conditions in Indigenous Australians. Currently Louise is the lead investigator on several large NHMRC-funded projects, including the Northern Territory and Far North Queensland Diabetes in Pregnancy Partnership and The eGFR study (Accurate assessment and progression of kidney damage in Indigenous Australians). After completing the majority of her physician and endocrinology training at St Vincents Hospital Sydney, Louise moved to Darwin in 2002 to pursue her passion for improving the health of Indigenous Australians. Louise is currently on the Australian Diabetes Society Council and was previously a member of the Council of the Australasian Diabetes in Pregnancy Society. Louise has been providing clinical diabetes services to urban and remote NT communities for over 15 years, including more recently via telehealth.

KEYNOTE SPEAKER

DR. JILL HAMILTON, MD

"Sweet Babies: Outcomes of Gestational Diabetes Exposure In utero"

Epidemiologic studies indicate children exposed to gestational diabetes in utero experience increased long term risk of obesity and type 2 diabetes. Less is known about how these processes evolve during childhood. This talk will focus on results from 2 cohort studies examining childhood adiposity and cardiometabolic risk in young children during the first few years of life, and in a second group of children studied at age 8-12 years.

BIOGRAPHY

Dr. Jill Hamilton is a Pediatric Endocrinologist at the Hospital for Sick Children, Senior Associate Scientist at the Research Institute, and Professor of Paediatrics at University of Toronto. Her clinical work is in endocrinology and diabetes with a particular focus in the areas of obesity and type 2 diabetes. Her research focuses on (i) the biologic and psychosocial determinants of obesity, including intra-uterine exposure to hyperglycemia, (ii) metabolic complications of obesity in childhood, and (iii) obesity treatment in children and adolescents.

KEYNOTE SPEAKER

DR. ELAINE URBINA, MD, MS

"Vascular and Cardiac Target Organ Damage in Diabetes"

The epidemiology of cardiovascular disease in adults with diabetes will be presented followed by data supporting the existence of sub-clinical vascular damage in youth with T1D and T2D. The relationship between vascular and cardiac damage will be discussed ending with intervention studies suggesting strategies to improve CV outcomes in patients with diabetes.

BIOGRAPHY

As Director of Preventive Cardiology at Cincinnati Children's Hospital Medical Center, Dr. Urbina's clinical activities focus on prevention (obesity, hypertension and dyslipidemias) while her research grants (AHA, NIH) and masters in epidemiology training concentrate on new non-invasive methods of assessing atherosclerotic CV target organ damage in youth related to CV risk factors especially those that cluster with obesity. She has over 25 years of experience in non-invasive imaging of CV structure and function in large epidemiologic studies such as the Bogalusa Heart Study. She was PI of a National Institutes of Health (NHLBI R01) following the cardiac and vascular effects of obesity and type 2 diabetes on adolescents and is currently a Co-PI of the International Childhood CV Cohorts Consortium grant that is completing 40-year follow-up on subjects enrolled as children in multiple cohorts including Bogalusa, Muscatine, Young Finns and the National Growth and Health study of which she is PI. She is also Director and PI of an AHA Strategically Focused Network in HTN grant that is exploring population, clinical and epigenetic determinants of target organ damage in hypertensive youth. Her CV core also supplies training for many multi-center pediatric studies including CKiDs (chronic kidney disease) and serves as the CV Imaging Core for a variety of cohorts including TODAY2 (type 2 diabetes), SEARCH 3 (type 1 diabetes), FUEL (single ventricle congenital heart disease), Do IT (dyslipidemia) and TIDES (environmental exposures).

KEYNOTE SPEAKER

DR. TIM KIEFFER, PhD

"Cell-Based Insulin Replacement for Diabetes "

This presentation will introduce the challenges with maintaining normal blood glucose levels with current therapies; review the effectiveness of islet cell transplant providing the rationale for generating islet cells from pluripotent stem cells, leading to the preclinical data that has supported recent clinical trials of encapsulated differentiated human stem cells in patients with type 1 diabetes.

BIOGRAPHY

Dr. Timothy Kieffer, PhD is a Professor in the Departments of Cellular & Physiological Sciences and Surgery and leader of the Diabetes Research Group in the Life Sciences Institute at the University of British Columbia, Vancouver. His laboratory (kiefferlab.com) is focused on the development of novel gene and cell therapy approaches to treat diabetes. He has co-authored >175 peer reviewed publications on these topics and received scholarships from the Canadian Diabetes Association, the Alberta Heritage Foundation for Medical Research, the Michael Smith Foundation for Health Research, and JDRF. In 2016 he spent 1 year on sabbatical at The Center for iPS Cell Research and Application (CiRA), Kyoto University.

KEYNOTE SPEAKER

DR. JOSEPH BASS, PhD

"Circadian Integration of Glucose Homeostasis with Sleep/Wake State"

The circadian clock is encoded by an intrinsic transcription feedback loop that synchronizes behavior and metabolism with the light cycle. 24-hr transcriptional cycles are present in nearly all mammalian cells and genetic studies have demonstrated a requirement for cell autonomous clocks in energy constancy. To determine how molecular clocks govern cell type-specific physiology, we have investigated pancreatic beta cell circadian regulation of signal dependent transcription. We observed time-of-day programming of active chromatin within beta cell enhancers driving rhythmic phases of transcriptional activation and repression. Using the assay of transposase-accessible chromatin sequencing, we have identified a requirement for the molecular clock in nucleosome positioning and chromatin opening. Chromatin dynamics across the light-dark cycle in turn determined the magnitude of stimulus-induced transcription. Epigenetic programming corresponded by amperometry with rhythmic circadian regulation of glucose-evoked capacitance and insulin release. Our studies reveal cell type-specific chromatin and transcriptional dynamics coordinating metabolism in anticipation of sleep.

BIOGRAPHY

JosDr. Joseph Bass is a Charles F. Kettering Professor of Medicine and Chief of the Division of Molecular Medicine and Endocrinology at Northwestern University. The major focus of his research is on the molecular integration of circadian and metabolic systems. A breakthrough stemmed from our studies of the first circadian mutant mice showing that these animals have altered sleep, feeding activity, obesity and diabetes (Science 2005). We subsequently showed that the clock system regulates peripheral cell functions including insulin secretion (Nature 2010) and mitochondrial respiration (Science 2013). We have also applied genomic approaches to identify cell type specific mechanisms of circadian regulation (Science 2015), and identified a mechanism through which the circadian cycle is coupled to the biosynthesis of NAD^+ (Science 2009, 2013). An overarching theme in our work relates to understanding the impact of metabolic milieu on behavior and endocrine physiology. We are poised to apply genetic manipulation in individual tissues of the mouse and also human cells to examine the role of clock control of mitochondrial function on sleep, circadian systems, and healthy aging.

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ORAL ABSTRACTS

MELISSA GABBS, University of Manitoba (POSTER #15)

HIGH RATES OF AMBULATORY AND NOCTURNAL HYPERTENSION IN YOUTH WITH TYPE 2 DIABETES

Melissa Gabbs, Brandy Wicklow, Jonathan McGavock, Elizabeth Sellers, Tom Blydt-Hansen, Kristine Kroeker, Dan Chateau, Farrah Jabar, Allison Dart for the iCARE Investigator Team.

Background: Youth with type 2 diabetes (T2D) are at high risk for developing cardiovascular complications. Recent guidelines from the American Academy of Pediatrics suggest screening for ambulatory hypertension in all youth with diabetes. However, little is known about 24-hour ambulatory blood pressures (ABPM) in youth with T2D.

Objectives: Describe ABPM findings of youth with T2D and identify the associations between ambulatory and nocturnal hypertension and early markers of cardiorenal morbidity.

Methods: A cross-sectional analysis was used to examine 24-hour ABPM among 195 youth with T2D from The *I*mproving renal *C*omplications in *A*dolescents with T2D through *RE*search (iCARE) study. Individuals were stratified into no hypertension, ambulatory, and nocturnal hypertension groups. Associations were evaluated between hypertension status, clinical risk factors, albuminuria and early cardiovascular risk [carotid intima media thickness (CIMT) and left ventricular mass index (LVMI)].

Results: The cohort had a mean age of 14.9±2.4 and a median diabetes duration of 2.0 (1.0,3.8) years, 67.7% were female. The majority of our cohort (69%, n=134) had hypertension on ABPM, and 48.2% (n=94) had isolated nocturnal hypertension. Hypertension was associated with elevated hemoglobin A1c (p=0.02), albuminuria (ACR >2mg/mmol; p=0.02) and increased CIMT (p=0.04). There were no differences in age at diagnosis, median duration of disease, BMIz score, or LVMI between groups.

Conclusions: ABPM identified high rates of ambulatory and nocturnal hypertension in youth with T2D. Associations were seen with both renal and cardiovascular outcomes, supporting their routine use in clinical practice.

STEPHANIE KERELIUK, University of Manitoba (POSTER #16)

EXPOSURE TO GESTATIONAL DIABETES ALTERS CARDIAC GENE EXPRESSION AND METABOLISM IN THE RAT OFFSPRING AND INDUCES CARDIAC DYSFUNCTION WITH AGE.

Stephanie M. Kereliuk, Prasoon Agarwal, Laura K. Cole, Kyle G. Cheung, Bo Xiang, Mario A. Fonseca, Grant M. Hatch, Jonathan McGavock, Vernon W. Dolinsky.

Introduction: Gestational diabetes mellitus (GDM) is the most common complication of pregnancy. Children exposed to GDM are at increased risk for cardiovascular disease development later in life, though the mechanisms responsible are unknown. We hypothesize that fetal exposure to GDM induces cardiomyocyte mitochondrial dysfunction, and left ventricular (LV) dysfunction with age.

Methods: GDM was induced in female rats with a high fat (45% kcal) and sucrose diet prior to mating, throughout pregnancy and lactation. Lean control females received a low fat (10% kcal) diet. LV morphology and function were assessed throughout the life course of the offspring (e18 to 12-months of age) by transthoracic echocardiography. Fetal rat ventricular cardiomyocytes (FRVC) were isolated from e20.5 offspring for mitochondrial respiration and calcium transport analysis. Serum metabolome and cardiac transcriptome profiles from 3-month old offspring were measured by LC-MS, and RNASeq.

Results: Offspring exposed to GDM exhibit increased LV posterior wall thickness across their life course (fetal to 12-months of age; $p<0.05$) and impaired LV filling beginning at 6-months of age ($p<0.05$). Consistent with in vivo diastolic dysfunction, alterations in calcium flux and sarcoplasmic reticulum-dependent calcium re-uptake (1.5-fold and 1.6-fold greater, respectively) were observed in FRVC from GDM offspring ($p<0.05$). RNASeq analysis revealed that 3-month old offspring exposed to GDM exhibit altered calcium handling gene expression (e.g. *ATP2B2* and *ATP2A3)*. Mitochondrial oxygen consumption was reduced for glucose, and fatty acid substrates in FRVC isolated from GDM offspring ($p<0.05$). Serum metabolomic analysis revealed that b-hydroxybutyrate levels are elevated (2.4-fold, $p<0.05$) and several citric acid cycle intermediates are reduced in 3-month old GDM offspring, indicative of altered mitochondrial ATP production.

Conclusion: GDM conditioned mitochondrial dysfunction, altered contractility, and calcium transporter gene expression in cardiomyocytes of the offspring, in concert with LV hypertrophy and diastolic dysfunction. Our findings identify several mechanisms that link early-life GDM exposure to cardiovascular disease development.

MYRIAM HOYECK, Carleton University (POSTER #17)

EFFECTS OF CHRONIC LOW-DOSE TCDD EXPOSURE DURING PREGNANCY ON OFFSPRING PANCREAS DEVELOPMENT AND FUNCTION; A POTENTIAL RISK FACTOR FOR INCREASED DIABETES SUSCEPTIBILITY

Myriam P. Hoyeck and Jenny E. Bruin

Background: Diabetes prevalence is increasing at exponential rates and epidemiological studies have shown a correlation between dioxin exposure and diabetes incidence. Dioxins are a group of highly persistent organic pollutants that show widespread global distribution. Preliminary data in the Bruin lab has shown that exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the most toxic dioxin, upregulates *Cyp1a1* expression (a biomarker of dioxin exposure) in islets, supresses glucose-stimulated insulin secretion in human and mouse adult β-cells, and causes β-cell loss in adult mice. Abnormalities in β-cell mass and function are key characteristics of diabetes, as such early life exposure to dioxins may confer a lifetime risk of developing diabetes. The purpose of this study was to analyse the effects of a chronic low-dose exposure to TCDD during pregnancy on offspring pancreas development and function.

Methodology: Female mice were treated with TCDD (20 ng/kg/d) or corn oil (vehicle) 2x per week prior to and throughout gestation, and during lactation. Plasma, liver, and pancreas were collected from offspring at birth and 3 weeks of age for analysis by ELISA, qPCR, and immunohistochemical staining. Long-term changes in pancreas function are being assessed using *in vivo* glucose-stimulated insulin secretion assays, and glucose and insulin tolerance tests.

Results: Neonates from TCDD-treated dams were found to have significantly decreased blood glucose levels, increased plasma insulin levels, upregulated *Cyp1a1/2* expression in liver and pancreas, and altered expression of markers of pancreas development and function. The decrease in blood glucose observed at birth was seen to persist until 3 weeks in TCDD-exposed male offspring, after which the males became hyperglycemic.

Conclusions: The results thus far suggest that early life exposure to TCDD may predispose offspring to defects in pancreas development and function, increasing diabetes risk. The long-term implications of this exposure are currently being investigated in both male and female offspring.

SIMONE daSILVA ROSA, University of Manitoba (POSTER #18)

THERAPEUTIC TARGETING OF SKELETAL MUSCLE NIX IN EARLY-ONSET INSULIN RESISTANCE

Simone da Silva Rosa, Matthew Martens, Jared Field, Donald Chapman, Christof Rampitsch, Vernon Dolinsky and Joseph Gordon

Introduction: Lipotoxicity is a form of cellular stress caused by the accumulation of lipids resulting in mitochondrial dysfunction and insulin resistance in muscle. Previously, we demonstrated that Nix is a lipotoxicity-responsive gene that accumulates in response to diacylglycerols induced by high-fat and sucrose (HFS) feeding and exacerbated by exposure to gestational diabetes (GDM) during fetal development. Here we identify a novel phosphorylation residue, activated by clenbuterol treatment that can prevent Nix-induced mitochondrial dysfunction in muscle cells.

Methods: C2C12 skeletal muscle myotubes were exposed to 200 μM palmitate, or vehicle control. To assess mitochondrial membrane potential, cells were stained with TMRM, and imaged through epifluorescence. Plasmid-based PKA biosensor was used to identify pharmacological PKA activation by clenbuterol and cilomilast. Cellular localization of Nix was determined by cell fractionation and protein expression by western blot. Phospho-peptide mapping was performed by mass spectrometry and custom phopho-specific antibody was generated. One-way anova determined multiple comparisons between groups and student t-test compared mean differences.

Results: In a series of gain-of-function and loss-of-function experiments in rodent and human myotubes, we demonstrate that Nix accumulation triggers mitochondrial depolarization ($p<0.05$), fragmentation ($p<0.05$), calcium-dependent activation of DRP-1, and mitophagy ($p<0.05$). In addition, Nix-induced mitophagy leads to myotube insulin resistance through activation of mTOR-S6K inhibition of IRS-1. Finally, we demonstrate that Nix-induced mitophagy and insulin resistance can be reversed by direct phosphorylation of Nix by PKA, leading to the translocation of Nix from the mitochondria and sarcoplasmic reticulum to the cytosol.

Conclusion: These findings provide insight into the role of Nix-induced mitophagy and muscle insulin resistance during an overfed state. Furthermore, our data supports the hypothesis that Nix regulates mitochondrial metabolism and insulin signaling in myotubes and suggest a mechanism by which pharmacological activation of PKA may circumvent the mitochondrial dysfunction characteristic of insulin resistance.

POSTER ABSTRACTS

POSTER #1

ALTERED METHYLATION OF PROMOTERS IN GENES INVOLVED IN INFLAMMATORY AND METABOLIC PATHWAYS IN FIRST NATIONS ADOLESCENTS WITH TYPE 2 DIABETES

Prasoon Agarwal, Brandy A. Wicklow, Allison B. Dart, Elizabeth Sellers, Wayne Xu, James R. Davie, and Vernon W. Dolinsky

Introduction: Type 2 diabetes (T2D) is increasing in adolescents. DNA methylation is a cellular process by which methyl groups are added to DNA molecules, regulating gene expression. Obesity may be associated with T2D but Indigenous populations are disproportionally affected by this complication and the cause is unknown.

Objective: We hypothesize that DNA methylation patterns of genes associated with obesity and T2D risk are altered in First Nations adolescents diagnosed with T2D.

Methods: Peripheral blood mononuclear cells (PBMCs) were obtained from a prospective cohort of First Nations adolescents diagnosed with T2D (n=21) and majority of whom are obese (the iCARE cohort) and the controls (n=10). All samples were sequenced using SOLiD. Data was analyzed using diffReps with a 200bp window size and the Negative Binomial test to identify differentially methylated regions (DMRs). A false discovery rate ≤ 0.05 and fold change ≥ 1.5 were used as cut-off parameters.

Results: 565447 DMRs were identified in PBMCs from T2D patient samples compared to controls. DNA from the PBMCs of First Nations adolescents with T2D were hypomethylated compared to controls. We identified several novel DMRs, of which 1110 were found in the gene promoter region. These gene promoter region DMRs included those associated with pro-inflammatory and metabolic pathways, including; glycoprotein hormones, alpha polypeptide (CGA), janus kinase 2 (JAK2), interleukin 5 (IL5), and glutamate-ammonia ligase (GLUL), glutamyl-tRNA amidotransferase subunit B (GATB), glutaminase 2 (GLS2), U2 small nuclear RNA auxiliary factor 2 (U2AF2) respectively.

Conclusions: Differences were observed in the DNA methylation pattern of PBMCs from First Nations adolescents diagnosed with T2D when compared to controls. Differential methylation of promoters in genes involved in inflammatory and metabolic pathways could play a crucial role in defining T2D risk in First Nations adolescents.

POSTER #2

INSULIN-LIKE GROWTH FACTOR-1 AUGMENTS MITOCHONDRIAL FUNCTION THROUGH AMPK TO DRIVE AXONAL REPAIR AND PROTECT FROM SENSORY NEUROPATHY IN TYPE 1 DIABETES

Mohamad-Reza Aghanoori, Darrell R. Smith, Shiva Levari-Shariati, Andrew Ajisebutu, Nigel A. Calcutt, Michel Aliani and Paul Fernyhough

Background: Diabetic sensorimotor polyneuropathy (DSPN) affects approximately half of diabetic patients leading to significant morbidity. There is impaired neurotrophic growth factor signaling, AMP-activated protein kinase (AMPK) activity and mitochondrial function in dorsal root ganglia (DRG) in animal models of type 1 and type 2 diabetes. We hypothesized that sub-optimal insulin-like growth factor 1 (IGF-1) signaling in diabetes contributes to loss of AMPK activity and mitochondrial function contributing to development of DSPN.

Methods: Age-matched control rats and streptozotocin (STZ)-induced type 1 diabetic rats with/without IGF-1 therapy were used for *in vivo* studies. For *in vitro* studies, DRG neurons from control and diabetic rats were cultured and treated with/without IGF-1 in the presence or absence of inhibitors or siRNAs.

Results: Dysregulation of mRNAs for IGF-1, AMPKα2, ATP5a1 (subunit of ATPase) and PGC-1β occurred in DRG of diabetic vs. control rats. IGF-1 up-regulated mRNA levels of these genes in cultured DRGs from control or diabetic rats. IGF-1 treatment of DRG cultures significantly ($P<0.05$) increased phosphorylation of Akt, P70S6K, AMPK and acetyl-CoA carboxylase (ACC). Mitochondrial gene expression and oxygen consumption rate (spare respiratory capacity), ATP production, mtDNA/nDNA ratio and neurite outgrowth were augmented ($P<0.05$). AMPK inhibitor, Compound C, or AMPKα1-specific siRNA suppressed IGF-1 elevation of mitochondrial function, mtDNA and neurite outgrowth. Diabetic rats treated with IGF-1 exhibited reversal of thermal hypoalgesia and corneal nerve profiles. In diabetic rats IGF-1 elevated AMPK and P70S6K phosphorylation, Complex IV-MTCO1 and Complex V-ATP5a proteins, and restored Complex IV and I enzymatic activities in the DRG. IGF-1 prevented TCA metabolite build-up in nerve.

Conclusion: In DRG neuron cultures IGF-1 signals via AMPK to elevate mitochondrial function and drive axonal outgrowth. *In vivo* we propose this signaling axis mediates IGF-1-dependent protection from distal dying-back of fibers in diabetic neuropathy.

POSTER #3

RESVERATROL SUPPLEMENTATION IMPROVES MATERNAL GLUCOSE TOLERANCE AND PREVENTS GESTATIONAL DIABETES-INDUCED CARDIOMETABOLIC DISEASE DEVELOPMENT IN THE RAT OFFSPRING

Gabriel M. Brawerman[1,2], Stephanie M. Kereliuk[1,2], Troy J. Pereira[1,2], Bo Xiang[1,2], Mario A. Fonseca[1,2], Vernon W. Dolinsky[1, 2]

[1]Diabetes Research Envisioned and Accomplished in Manitoba (DREAM) Research Theme of Children's Hospital Research Institute of Manitoba, [2]Department of Pharmacology and Therapeutics, University of Manitoba, Winnipeg, MB, Canada

Introduction: Gestational diabetes mellitus (GDM), which affects 5-10% of pregnancies, is characterized by hyperglycemia in the third trimester of pregnancy. GDM increases cardio-metabolic disease risk in the offspring. Resveratrol (RESV), a naturally produced polyphenol, has anti-oxidant properties and positive metabolic health effects. We hypothesize that RESV administration (150 mg/kg) to pregnant GDM dams in the third trimester will improve maternal glucose tolerance and protect offspring from GDM-induced obesity and heart disease.

Methods: Six weeks prior to mating, female Sprague-Dawley rats consumed a high fat and sucrose (HFS) diet (45% kcal fat) to induce GDM, while lean control females received a low fat (LF) diet (10% kcal fat). In the third trimester, a subgroup of pregnant HFS-fed rats were supplemented with RESV (150 mg/kg). After weaning, offspring were randomly assigned a HFS or LF diet for 12 weeks. Offspring lipid levels were analyzed by ELISA and dual energy X-ray absorptiometry scans were used to assess lean and fat body mass. Echocardiography was used to assess cardiac function and morphometry.

Results: RESV improved maternal glucose tolerance, without affecting maternal body weight. In the neonatal offspring, RESV prevented GDM-induced elevated body and heart weights ($p<0.05$). GDM induced obesity in 15 week-old offspring and obesity was prevented in offspring exposed to GDM+RESV ($p<0.05$). Liver, cardiac, and circulating triglycerides were reduced in GDM+RESV offspring versus GDM offspring ($p<0.05$). Consumption of HFS by the offspring increased fat mass and percent body fat in all groups of offspring ($p<0.05$). GDM+RESV offspring exhibited similar heart weights to that of lean offspring but had reduced left ventricular posterior wall thickness against GDM offspring ($p<0.05$). Functional parameters were similar in all groups.

Conclusion: Maternal RESV supplementation during the third trimester of pregnancy prevented GDM-induced obesity, cardiac hypertrophy, and cardiac and hepatic steatosis in the offspring.

POSTER #4

ALTERED ISLET FUNCTION MAY PROMOTE A LEAN PHENOTYPE IN TAFAZZIN-DEFICIENT MICE

Laura K. Cole[1]*, Christine Doucette[1], Marilyne Vandel[1], Mario Fonseca[1], Bo Xiang[1], Genevieve C. Sparagna[2], Vern W. Dolinsky[1], Grant M. Hatch[1]

[1] Children's Hospital Research Institute of Manitoba, Faculty of Health Sciences, University of Manitoba, Winnipeg, Canada
[2]Department of Medicine, University of Colorado Denver, Anschutz Medical Campus, Aurora, USA.

Barth syndrome (BTHS) is a rare x-linked genetic disease. Mutations occur in the gene tafazzin which causes the content and molecular structure of cardiolipin to be altered in the inner mitochondrial membrane. A well-established characteristic of BTHS is growth deficiency. Previous evaluations of individuals with BTHS have indicated low-mean body weights. In this study, we utilized a mouse model with an inducible tafazzin shRNA knock-down to investigate the *in vivo* effects of tafazzin deficiency on weight gain. Previously, we have established that tafazzin knock-down mice were lean and maintained insulin sensitivity compared to the obese insulin resistant control litter mates. We have now determined that tafazzin protects against elevation in islet insulin content and loss of glucose-induced insulin secretion which are often associated with obesity-induced insulin resistance. We have ascertained that the quantity of beta-cells was similar between genotypes. However, insulin secretion during basal conditions was reduced from islets isolated from tafazzin knock-down mice. As a result, tafazzin knock-down mice exhibited significantly reduced basal insulin plasma levels. Preliminary data suggests that mitochondrial oxygen consumption is elevated due to increased heat production in islets lacking tafazzin. Our experiments indicate that tafazzin may have a role in regulating islet beta-cell function. These data also suggest that mice deficient in tafazzin may gain less weight, in part, by reducing basal insulin levels. (Supported by HSFC, BSF Canada/USA and the IMPACT training program).

POSTER #5

INCREASED PHYSICAL ACTIVITY PATTERNS ABOVE CURRENT GUIDELINES DOES NOT INCREASE GLUCOSE VARIABILITY IN TYPE 1 DIABETES

Nika Korpesho, BKin[1,2]; Andrea MacIntosh[2], MSc; Jacqueline Hay[1,2,3], MSc; Jane Yardley[4], PhD; Normand Boulé[4], PhD; Dessi P. Zaharieva[5], MSc; Michael C. Riddell[5], PhD; Jonathan McGavock[1,2], PhD

[1]University of Manitoba; [2]Children's Hospital Research Institute of Manitoba; [3]St. Boniface General Hospital Albrechtsen Research Centre; [4]University of Alberta; [5]York University

High glucose variability (GV) has been associated with hypoglycemia in persons with type 1 diabetes (T1D), which may result in coma or death. The role of daily physical activity (PA) for overnight and next-day glucose control is poorly understood. A multi-site observational study was undertaken to observe the impact of achieving moderate-vigorous physical activity (MVPA) guidelines on glycemic profiles. Twenty-five participants (15 female; 25±6 years; HbA_{1c} 7.6±1.1%; duration of diabetes 9±11 years) wore continuous glucose monitors (CGM) and accelerometers for six days. Participants yielded 125 independent days of PA and CGM data, which were stratified into three groups: (1) Below guideline days – did not achieve 30 minutes of MVPA/day; (2) Meeting guideline days – accumulated 30-59.9 minutes MVPA/day and (3) Training days – achieved ≥60 minutes MVPA/day. GV was calculated using Continuous Overall Net Glycemic Action (CONGA) and Mean Absolute Glucose (MAG) change, for both overnight and next-day periods. No significant differences were observed between below guidelines, meeting guidelines and training days for mean glucose (8.4±2.9, 8.5±2.6, 8.8±3.1 mmol/L, respectively, p=0.58), overnight MAG (2.5±1.0, 2.4±1.1, 2.3±0.9, p=0.79), or overnight CONGA-4 (2.1±1.7, 2.0±1.1, 2.0±1.3, p=0.65). These differences remained insignificant when comparing next-day values (mean glucose p=0.86, MAG p=0.18, CONGA-4 p=0.34). Achieving or exceeding daily MVPA targets was not associated with better glucose control or reduced GV in persons with T1D; however, further investigations with a larger sample size and various exercise modalities is warranted.

POSTER #6

MISOPROSTOL REGULATES BNIP3 ACTIVITY IN THE HEART TO PREVENT HYPOXIA-INDUCED NEONATAL CARDIOMETABOLIC DYSFUNCTION

Matthew Martens, Jared Field, Wajihah Mughal, Simone da Silva Rosa, Tammy Ivanco, William Diehl-Jones, Joseph Gordon

Children's Hospital Research Institute of Manitoba, John Buhler Research Centre, Health Sciences Centre, Winnipeg, Manitoba

Introduction: Systemic hypoxia affects more than 60% of preterm infants and is associated with both impaired cardiac metabolism and the development many diseases of prematurity. While the mechanism of injury remains elusive, it is clear that the hypoxia-inducible death gene, Bnip3, plays a central role. We hypothesize that with the addition of prostaglandin signaling through Misoprostol, the cellular activity of Bnip3 can be inhibited, thereby protecting the neonatal heart from hypoxia-induced cardiometabolic dysfunction.

Methods: Cardiometabolic dysfunction was assessed in primary neonatal cardiomyocytes that were exposed to environmental hypoxia (1% oxygen) and treated with Misoprostol. Following treatment, both mitochondrial membrane potential and free radicle production were measured via fluorescence microscopy (n=3). Concurrently, flow cytometry was used to determine cardiomyocyte viability, which was compared to control treatments [normoxia (21% oxygen) and/or drug vehicle] (n=3). To explore the underlying mechanism, a cardiac myoblast cell line (H9c2) was used in gain-of-function transfection experiments in combination with epiflorescent imaging and biochemical assays.

Results: In primary neonatal cardiomyocytes, environmental hypoxia reduced mitochondrial membrane potential by more than 61% and caused a 47% increase in cytotoxic free radicle production ($p<0.01$). These observed changes in mitochondrial function were also accompanied by significant reductions in cardiomyocyte viability ($p<0.01$). Importantly, all three measures of myocyte function were restored to control levels with the application of Misoprostol ($p<0.01$). Consistent with the hypoxia studies, Bnip3 expression in H9c2 cells had significant deleterious effects on mitochondrial structure and membrane potential, as well as cellular viability ($p<0.01$), which were all blocked with the addition of Misoprostol ($p<0.01$). However, when a functionally important Bnip3 phosphorylation-motif was neutralized, and when PKA was inhibited, Misoprostol-induced cardiometabolic protection was completely lost ($p<0.01$).

Conclusions: This work demonstrates that prostaglandin-induced modulation of Bnip3 is advantageous to cardiomyocyte protection and may further serve to prevent hypoxia-induced cardiometabolic dysfunction in the neonatal heart.

POSTER #7

INVESTIGATING THE CONTRIBUTION OF THE HNF-1αG319S GENE VARIANT TO EARLY-ONSET TYPE 2 DIABETES USING MOUSE ISLETS AND MIN6 β-LIKE CELLS

Taylor S. Morriseau[1,2], Tianna N. Flett[1], Kristin L. Hunt[1,3], Mario A. Fonseca[1,2], Prasoon Agarwal[1,2], Cuilan Nian[4], Vernon W. Dolinsky[1,2], Francis Lynn[4], Christine A. Doucette[1,2,3]

[1]Diabetes Research Envisioned and Accomplished in Manitoba (DREAM) Theme, Children's Hospital Research Institute of Manitoba (CHRIM), [2]Pharmacology & Therapeutics, [3]Physiology & Pathophysiology, University of Manitoba, [4]BC Children's Hospital Research Institute, Vancouver, BC

Background: 40% of Manitoban Indigenous youth with T2D harbor the HNF-1αG319S variant; the strongest genetic predictor of this disease currently known. Despite clinical evidence implicating pancreatic β-cell defects under post-colonial dietary stress, the role of HNF-1αG319S in β-cell dysfunction remains unknown. We hypothesize the G319S variant alters β-cell metabolism and insulin secretion depending on nutrient availability.

Methodology: To create appropriate models to test our hypothesis, CRISPR/Cas9 was used to knock-in the G>A.955 single nucleotide substitution into clonal MIN6 β-cells ("G319S-MIN6") and a C57/BL6 mouse model. Glucose-stimulated insulin secretion (GSIS) assays were performed on MIN6 β-cells (N=6) and isolated islets (N=3) from 3-month male wildtype and heterozygous mice. Follow-up *in vitro* measurements included qPCR (N=6) and GSIS following 24-hour exposure to 0.25mM palmitate (N=3).

Results: HNF-1αG319S did not affect GSIS in MIN6 β-cells (8.73 vs. 8.91 ng insulin/μg DNA/hr) or isolated islets (1.76 vs. 1.98 ng insulin/μg DNA/hr); however, basal insulin secretion decreased 3.2-fold relative to WT-MIN6 and 1.8-fold relative to wildtype mice. Metabolic gene expression was altered in G319S-MIN6, including a 4-fold downregulation in glucokinase (glucose metabolism) and a 2-fold upregulation in carnitine palmitoyltransferase-1A (mitochondrial fatty-acid uptake). With a seemingly increased capacity to shuttle fatty acids towards beta-oxidation, G319S-MIN6 cells maintained 15-fold GSIS under chronic lipotoxic stress, which otherwise severely impaired WT-MIN6.

Conclusion: HNF-1αG319S alters the gene expression profile of MIN6 β-cells. Surprisingly, the HNF-1α variant does not impair GSIS in MIN6 β-cells or islets from heterozygous mice, and may even confer resistance to palmitate-induced impairments in GSIS. Rather, G319S-expressing MIN6 β-cells and islets display reduced basal insulin secretion that although beneficial for maintaining whole-body glucose homeostasis during fasting, may trigger hyperglycemia when combined with excessive carbohydrate intake in the fed state. Future studies will address whether reduced dietary glucose and increased lipid consumption protects against T2D in G319S-expressing mice.

POSTER #8

ADIPONECTIN DEFICIENCY LEADS TO HEPATIC STEATOSIS AND GESTATIONAL DIABETES IN PREGNANT MICE

Brittany L. Moyce, Laura K. Cole, Bo Xiang, Mario A. Fonseca, Christine A. Doucette, Grant M. Hatch and Vernon W. Dolinsky

Introduction: Gestational diabetes mellitus (GDM) is a common pregnancy-related condition with implications for maternal and neonatal health. Factors such as diet and genetics contribute to development GDM, but evidence suggests that low levels of adiponectin are correlated with elevated risk. Adiponectin is a fat derived hormone that improves insulin sensitivity. We hypothesize that adiponectin deficiency causes fatty liver during pregnancy that induces GDM.

Methods: We compared the insulin insensitivity of low fat and high fat and sucrose diet fed pregnant (3rd trimester) adiponectin$^{-/-}$ (strain B6;129-*Adipoq*^{tm1Chan}/J) and wild-type mice. We assessed parameters of hepatic metabolism, mitochondrial function and fatty acid metabolism. Adiponectin was added back to pregnant dams at the end of the second trimester by administering adenovirus expressing full-length adiponectin .

Results: In the third trimester, fasting pregnant adiponectin$^{-/-}$ are hyperglycemic even on a low-fat diet (9.2mmol/L vs. 7.7mmol/L in controls, $p<0.05$); they also display impaired glucose and insulin intolerance relative to wild-type controls. Pregnant adiponectin$^{-/-}$ mice developed hepatic steatosis, including a 3-fold elevation in hepatic triglycerides ($p<0.05$) relative to wild-type. Additionally, a 2.5-fold increase in hepatic fatty acid synthase and increased beta-hydroxybutyrate dehydrogenase expression ($p<0.05$) and a 2-fold increase in circulating ketones ($p<0.05$) was observed. A 2-fold reduction ($p<0.05$) in maximal mitochondrial respiration was measured in hepatocytes of pregnant adiponectin$^{-/-}$ mice. Despite increased fatty acid uptake, hepatocytes of adiponectin$^{-/-}$ mice exhibit elevated synthesis and secretion of triglycerides and cholesterol. Adiponectin supplementation to pregnant adiponectin$^{-/-}$ mice improved glucose tolerance, prevented fasting hyperglycemia, and attenuated fatty liver.

Conclusion: Adiponectin deficiency is associated with altered hepatic lipid metabolism and hepatic steatosis during the period of pregnancy associated with increased fat oxidation. Consequently, adiponectin deficiency dysregulated maternal insulin sensitivity and resulted in hyperglycemia that is characteristic of GDM. Adiponectin supplementation rescues the effects of adiponectin deficiency on insulin sensitivity and hepatic lipid metabolism.

POSTER #9

DNA METHYLATION MARKS IN NUCLEAR AND MITOCHONDRIAL DNA

Fadumo Osman [1 2], Ruey-Chyi Su[3], James R. Davie [1 2 4]

1. Children's Hospital Research Institute of Manitoba, 2. Department of Biochemistry and Medical Genetics, University of Manitoba, 3. National Microbiology Laboratory, 4. CancerCare Manitoba, Winnipeg, MB, Canada

Background: Epigenetic mechanisms regulate biological processes from conception to death. DNA methylation is the most extensively studied epigenetic modification with 5-methylcytosine (5-mC) and 5-hydroxymethylcytosine (5-hmC) being key epigenetic players. Interestingly, mitochondrial DNA (mtDNA) is also believed to be modified by both 5-mC and 5-hmC; nevertheless, the presence and function of such modifications are still highly debated. Moreover, mtDNA cellular content differs across tissue types depending on several factors. However, the ratio of mitochondrial DNA relative to the nuclear genome in leukocytes (CD4+, CD8+ T cells) is not well studied. Peripheral blood mononuclear cells (PBMCs) are frequently used in DNA methylation studies and considered an informative study model with CD4+ and CD8+ T cells being reported as "most distinctly poised for rapid methylome response to physiological stress and disease". Our objective is to investigate the differentially methylated and hydroxymethylated regions in nuclear and mitochondrial DNA of CD4+ and CD8+ T cells and the relative ratio of mitochondrial/nuclear genome in these cells to estimate whether the changes in methylation could possibly be contributed to changes in their mitochondrial DNA content.

Methodology: Whole blood samples were collected from healthy donors and PBMCs were sorted into CD4+ and CD8+ T cells for analyses of 5-mC and 5-hmC in total cellular DNA. MethylMiner protocol and hydroxymethylated DNA immunoprecipitation were used to enrich for methylated and hydroxymethylated DNA fragments.

Results: The samples are being sequenced and bioinformatic analysis for the nuclear and mitochondrial methylome will be done

Conclusion: Significant variations in the DNA methylation levels between those cells are predicted to be present and might likely be influenced by their relative nuclear / mitochondrial DNA ratio. Overall, this will broaden our understanding of the cell-type-specific epigenetic events in normal physiological state which in turn will impact the future epigenetic analysis of diseases such as diabetes.

POSTER #10

STANDARDIZED EXTRACT OF *CASSIA FISTULA* STALK (CFS) MODULATES HEALING PROCESS THROUGH INCREASED ANGIOGENESIS AND REPRESENTS A POTENTIAL THERAPEUTIC STRATEGY TO TREAT DIABETIC WOUNDS

Taiana Magalhães Pierdoná, Tamiris de Fatima Goebel de Souza, Marilia de Oliveira Nunes, Fernanda Soares Macedo, Alexia Nathália Brígido Assef, Diego Veras Wilke, Nylane Maria Nunes de Alencar.

Nucleus of Research and Development of Medicines, Department of Physiology and Pharmacology, Federal University of Ceará (UFC), Brazil.

Introduction: Ischemic vascular diseases and wounds frequently affect patients with Diabetes Mellitus. Decreased vascular endothelial growth factor (VEGF), prolonged inflammation, and increased oxidative stress contribute to deterioration of tissue repair in diabetic patients. Therefore, research focusing on anti-inflammatory and antioxidant agents that promote angiogenesis is an important strategy to improve wound healing. *Cassia fistula* is a common specie in Brazil and it has been linked to hypoglycemic activity. We hypothesize that CFS play an important role in modulating the healing process.
Methods: CFS or vehicle control was used to confirm its ability to promote protection of L929 fibroblasts induced to oxidative stress by H_2O_2. Measurement of growth factors and angiogenesis (TGF-β and VEGF) was also performed. To elucidate the anti-inflammatory effect in RAW 264.7 macrophages, NO, TNF-α, IL-1β and IL-6, ELISA assay was used. To assess proliferation, HACAT keratinocytes were subjected to MTT or SRB assays, as well as submitted to Scratch and flow cytometer. One-way ANOVA determined comparisons between multiple groups and student t-test compared mean differences.
Results: CFS showed a significant increase in viability through mitochondrial metabolism ($p < 0.05$) but did not increase fibroblast proliferation. CFS was also able to increase VEGF after 24h incubation ($p < 0.05$) (picograms/mL: vehicle: 3828 ± 48.4; CFS 100: 4422 ± 158.7) and protect cells from oxidative stress induced by H_2O_2 (% viability: vehicle: 15.57 ± 0.65, CFS 100: 23.37 ± 0.83). In addition, CFS increased keratinocyte proliferation and decreased NO and IL1-β in macrophages ($p < 0.05$).
Conclusion: These findings highlight the importance of fibroblasts, macrophages and keratinocytes in the healing process and suggest that CFS is able to decrease oxidative stress, decrease proinflammatory cytokines, increase viability, proliferation, and VEGF. Therefore, CFS represents a potential therapeutic strategy that may contribute in the treatment of diabetic complications characterized by poor neovascularization and persistent inflammation.

POSTER #11

THE NEXT GENERATION STUDY: AN UPDATE ON THE BIOLOGICAL RISK FACTORS FOR TYPE 2 DIABETES IN INDIGENOUS CHILDREN IN MANITOBA

Mae Santos, Farrah Z. Jabar, Christy Pylypjuk, Elizabeth A. C. Sellers and Brandy A. Wicklow

Background: Rates of early onset type 2 diabetes in Manitoba are ~20 times higher than other provinces in Canada, disproportionately affecting Indigenous populations (Amed et. al, 2010). The HNF1α G319S mutation found in the Oji-Cree population (Hegele et.al, 1999) increases the risk for youth-onset T2D by decreasing insulin (Sellers et. al, 2012). The Next Generation longitudinal birth cohort study aims to determine pre- and perinatal modifiable risk factors of T2D in children, and how risk factors interact with HNF1a status to inform interventions for at-risk children.

Methodology: The 'Next Generation' study follows children born in Manitoba to an Indigenous parent diagnosed in childhood with T2D. Mothers' anthropometric and biochemical characteristics during pregnancy and cord blood for HNF1a genotyping, epigenetic analysis, inflammatory markers and anthropometric measures of the infant are collected. Questionnaires about stressors and home environment during pregnancy are asked. Offspring's clinical and biochemical measures are completed: blood pressure, waist circumference, glycosylated hemoglobin and glucose tolerance test are done biennially from year 7.

Results: The cohort currently has 254 children and 133 parents (126 mothers, 7 fathers). 59/183 children were born prematurely (<37 weeks), and 22 resulted in congenital anomalies. 38 (15%) children have T2D, with the median age of diagnosis 11.0 years, interquartile range= 3.4 years. majority of those diagnosed carry one or two copies of the HNF1α G319S allele and are overweight/obese. Of these children HNF1α G319S status was S/S (n=8, 21%), S/G (n=22, 58%), and G/G (n=6, 16%).

Conclusions: The rates of childhood-onset T2D in our cohort is much higher than reported national rates (1.54/100,000 children/year) (Amed et al., 2010). Offspring who developed T2D were more likely to carry one or two copies of the HNF1α G319S alleles, indicating that a combination of genetics and in-utero exposure to T2D are involved in the development of childhood T2D.

POSTER #12

THE CIRCADIAN CLOCK REGULATES RHYTHMIC EXPRESSION OF UCP2 IN B CELLS AND CONTROLS DAILY CYCLES OF INSULIN SECRETION

Nivedita Seshadri[1,2], Michael Jonasson[2], Tianna N Flett[1,2], Youstina Soliman[1,2], Yilin Tian[1,2], Vernon W. Dolinsky[2,3], Christine A. Doucette[1,2]

[1]Univerisity of Manitoba, Department of Physiology and Pathophysiology, [2]The Children's Hospital Research Institute of Manitoba, Diabetes Research Envisioned and Accomplished in Manitoba (DREAM) Theme, [3]University of Manitoba, Department of Pharmacology & Therapeutics

Background: Chronodisruption is strongly associated with Type 2 Diabetes (T2D), a condition triggered by β-cell failure[1]. Healthy β-cells secrete insulin rhythmically over 24hrs to maintain glucose homeostasis[2,3]. We recently demonstrated that *Ucp2* expression is rhythmic over 24hrs and contributes to daily cycles of insulin secretion; however, the cellular and molecular mechanisms that regulate temporal *Ucp2* expression remain undefined[4].

Methods: To investigate if the β-cell circadian clock machinery regulates *Ucp2* expression, *Bmal1*, was silenced in synchronized MIN6 clonal β-cells using siRNA. Using this model of β-cell circadian dysfunction, we examined the impact on *Ucp2* mRNA expression, GSIS and ATP production. Furthermore, to determine if BMAL1 directly binds to the *Ucp2* promoter in a time-dependent manner, we performed chromatin immunoprecipitation (ChIP) on synchronized MIN6 cells. Interaction between BMAL1 protein and the *Ucp2* promoter was tested by qPCR using primers designed across the E-Box elements in the *Ucp2 and Nrf2* (positive control) promotor regions.

Results: Bmal1siRNA silencing impaired GSIS via a 2.4-fold upregulation of *Ucp2* expression at 4hrs post-synchronization compared to control cells. Increased *Ucp2* expression was associated with reduced ATP production at 4hrs indicative of chronically increased mitochondrial uncoupling. In our ChIP studies, qPCR revealed no enrichment of *Ucp2* at either time point, suggesting that BMAL1 does not directly bind to the *Ucp2* promoter.

Conclusion: The circadian clock regulates daily cycles of *Ucp2* expression in MIN6 cells, which is part of an important metabolic switch that aligns GSIS capacity with the time of day. Our ChIP studies suggest that rhythmic *Ucp2* expression is ***not directly*** regulated by the circadian clock; however, indirect regulation may occur through other circadian-controlled transcriptional regulators. In future, we will perform ChIP-seq to explore novel regulators of temporal *Ucp2* expression. Additionally, we will generate β cell-specific *Bmal1* knockout mice to examine the impact of these cycles in whole animals.

POSTER #13

LONG-TERM EFFECTS OF A PRE-NATAL LIFESTYLE INTERVENTION

H. TEKLEMARIAM, A. HUI, G. SHEN

Introduction: Previous prenatal lifestyle intervention studies demonstrate that participants who receive the lifestyle intervention tend to achieve healthier pregnancies compared to women who receive standard care (control group). However, there are less conclusive studies delineating the long-term health effects of those interventions.

Methodology: Participants enrolled in a previous prenatal lifestyle intervention study from 2004-2010 at the University of Manitoba have been contacted to assess if the previous intervention study has influenced their current lifestyle. A questionnaire was developed to determine the health status of the participants, and was forwarded to all consenting participants. Participants were given an in-person or phone explanation of the study. Nutrient intake was assessed via validated 3-day food record, and physical activity levels were assessed via PARmed-X Pregnancy Questionnaire. All assessment tools are consistent with previous methodology.

Results: Excessive gestation weight gain (EGWG) during index pregnancy in participants in both control and intervention groups was positively correlated with a BMI 30 or higher during follow-up ($R=0.31$, $p=0.03$). Participants in the control group were found to have a positive correlation between GWG and weight gain since index pregnancy ($R=0.51$, $p=0.02$). Participants in the intervention group also reported higher levels of physical activity, lower BMI, lower daily calorie intake, total fat and saturated fat intake (not statistically significant).

Conclusion: These results suggest that EGWG has a sustained impact on future weight gain, and that participants who received a lifestyle intervention during pregnancy may have positive long-term impact on their diet and activity.

POSTER #14

CARDIAC SIRT3 ATTENUATES DOXORUBICIN-INDUCED ALTERATIONS OF THE MITOCHONDRIAL ACETYLOME AND CARDIAC DYSFUNCTION IN RODENTS.

Mateusz M. Tomczyk, Bo Xiang, Stephanie M. Kereliuk, Kyle G. Cheung, Vernon W. Dolinsky

Introduction: Anthracyclines such as doxorubicin (DOX) are effective chemotherapeutics, but have limited application due to dose-dependent, cardiotoxic effects. Sirtuins are a class of lysine deacetylases that remove acetyl-groups from histones, proteins and metabolites. Previously our lab has shown that the expression of the mitochondrial Sirtuin 3 (SIRT3) is downregulated by DOX in the mouse heart.
Objective: We hypothesize that DOX increases mitochondrial protein acetylation via reduced SIRT3 expression and cardiac function in mice can be improved by increasing SIRT3 expression following treatment with DOX.
Methods: Mice expressing full length (M1-SIRT3) by the muscle creatine kinase promoter, and truncated (M3-SIRT3) by the alpha-myosin heavy chain promoter were used in this study and compared to non-transgenic mice. Mice were given DOX injections of 8.0mg/kg body weight for 4 weeks while controls received an equal volume of saline. Transthoracic echocardiography was performed on all mice (n=6 per group). Parameters of cardiac structure (e.g. left ventricular posterior wall thickness and internal dimensions), systolic and diastolic function (e.g. ejection fraction and intraventricular relaxation time, respectively) were measured. Mitochondria were isolated from the heart and tryptic digested peptides enriched for lysine acetylation with an anti-acetylated lysine antibody, were used for mass spectrometric analysis.
Results: DOX decreased left ventricular posterior wall thickness and intraventricular sepal thickness compared to controls ($P<0.05$), while M1-SIRT3 and M3-SIRT3 expression prevented cardiac remodelling. DOX reduced ejection fraction and increased intraventricular relaxation time in non-transgenic mice ($P<0.05$). SIRT3 transgene expression in the heart conferred resistance to DOX-induced functional impairments and maintained normal ejection fraction and intraventricular relaxation time ($P<0.05$). Initial mass spectroscopy data shows enrichment of acetylated peptides from metabolic enzymes in cardiac mitochondria.
Conclusion: Increased SIRT3 expression in the heart rescues DOX-induced cardiac dysfunction. Future mass spectrometric analysis will reveal how DOX alters mitochondrial protein acetylation and affects cardiac function.

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